The Investigation of Callus Induction and Indirect Regeneration in Kurdistan Colony Strawberry (*Fragaria×ananassa Duch*.)

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Abstract: In order to investigate callus induction and indirect regeneration of Kurdistan colony strawberry in geographical regions of Sanandaj, Kamyaran, and Mariwan, a factorial experiment in a Completely Randomized Designs (CRD) with three-replication was conducted. Plant growth regulators reused in callus induction and direct regeneration were: [BAP (10mg/l) + NAA (0.01mg/l)], [TDZ (1mg/l) + 2,4- D (0.2mg/l), TDZ (2.65mg/l) + IBA (0.01mg/l)], and MS medium (as control). Also, for indirect regeneration, they include: [BAP (2mg/l) + NAA (0.1mg/l)] [BAP (2mg/l) + IBA (2mg/l)] and MS medium (as control). Young leaves as well as meristem of lateral buds of young stolons were used as explants. At callus induction stage, percentage, wet weight, dry weight, transparency, continuity, color, stiffness, bump, and diameter of callus were measured in days 17, 23, 29, and 35. At direct regeneration stage, percentage and length of stem were measured in days 14, 21, 28, and 35. Also, both length of root in day 35 and growth rate of regeneration were measured. Finally, in indirect regeneration the number of regenerated cells, the growth rate of regeneration, the length of stem in days 16, 22, 28, 34, and 40 and the length of root in day 40 were measured as well. Leaf explants of three regions were completely destroyed in four Plant growth regulators. In terms of callus induction, only meristem explants of Mariwan region induced callus in Plant growth regulators of [BAP (2mg/l) + NAA (0.1mg/l)]. In terms of direct regeneration, the effect of regions in four Plant growth regulators was assessed and Mariwan region produced the best results in Plant growth regulators of [TDZ (2.65mg/l) + IBA (0.01mg/l)]. In terms of indirect regeneration, the Plant growth regulators of [BAP (2mg/l) + NAA (0.1mg/l)] and [BAP (2mg/l) + IBA (2mg/l)] were identified as the best Plant growth regulators treatment for regeneration percentage, the number of regenerated cells, basic MS medium (without hormone), and other studied traits. The significant difference among collected samples from Mariwan, Sanandaj and Kamyaran regions in terms of callus induction, indirect and direct regenerations indicated genetic diversity of Kurdistan colony.

Keywords: Strawberry, callus induction, regeneration, growth regulators, explants.

Introduction

Strawberry (*Fragaria vesca*) is a perennial plant from Rosacea family with stem and a universal popular fruit [1]. According to FAO statistics, Iran, in terms of strawberry cultivation area (including 2400 hectares, around 1%, out of 241109 hectares worldwide, takes the 19th place in the global ranking. The rate of strawberry production in the world is approximately 4,516,810 tons. Iran's share is about 32 thousand tons, which has taken the 21st place in the world (0.71% of the total world production). In Asia, cultivation and production rates are 32083 hectares and 829,223 tons, respectively. After Turkey, South Korea, and Japan, Iran took the fourth place, cultivating 7.48% of Asia's share and producing 3.86% of total Asia's production [2]. Strawberry is naturally proliferated through surface runner stem (stolon) and plant division [3]. Besides the traditional ways of proliferation, micro-propagation techniques could be used to proliferate or to

achieve eugenic objectives in strawberry. In this regard, Nahara *et al* (1990) reported that strawberry could be regenerated through cell suspension [4]. In terms of strawberry cultivars, some more studies have been carried out on different organs along with the use of Plant growth regulators with different concentrations [4]-[8]. Although over 62% of the country's strawberry is produced in Kurdistan Province, 100% of utilizable transplants inside the province and even in the whole country are still produced by traditional method, which is considered as the principal reason of yield (in unit area) reduction in province and country's farms. Not only micro-propagation techniques development is regarded as an essential part of in vitro breeding research programs and gene transfer to plants, but also, due to lack of dependency to season, high proliferation speed, and high quality, it is likely to produce disease-free plants and prevent many migratory diseases transmission through soil and plant [9] - [10]. Today, regeneration through tissue culture techniques is the foundation of many genes transfer methods as well as plant breeding. Plant regeneration from native tissue could be done using different ways. In the simplest way, known as direct regeneration, a small piece of plant is separated as explants and the given organ is formed in sterile conditions in the medium that contains growth regulators in appropriate concentrations. This kind of regeneration is without passing through the callus formation [11]. Biswas *et al* (2010), investigating callus tissue culture from 3 explants (leaf, rounded blade, and aerial root pieces) of strawberry reported that the combination of 4milligrams of NNA and 1.5mg/l of IBA gives the highest percentage of callus induction among all explants types [12]. Also, examining tissue culture of strawberry, Passey *et al*., (2003) found that leaf parts of strawberry have the highest callus induction and regeneration compared to of petiole [13]. Khan & Spoor (2000), by examining strawberry tissue culture reported that combining 2.25 mg/l of BA, 0.18 mg/l of NAA, and 1mg/l of IBA lead to the highest amount of callus induction in strawberry explants [8]. Campton & Gray (1995) compared different growth regulators such as Kinetin, BA, indole acetic acid and gibberellic acid at the stage of final establishment of the shoots of grapes and concluded that in the medium containing BA, more explants formed shoots compared to three other growth regulators [14]. They reported that the most shoot induction at MS medium contained 0.91 µM of TDZ and 0.98 µM of IBA; medium shoot induction at MS medium contained 0.91 µM of TDZ and 0.98 µM of IBA, and rooting at medium MS contained 3.22 µM of NAA. Considering the importance of strawberry, especially the valuable colony of Kurdistan in terms of regional compatibility and its favorable quality regarding taste, the current study carried out to investigate the rate of callus induction and indirect regeneration of meristem explants and leaf of Kurdistan colony strawberry. The study also investigated the diversity of collected plants from different regions in terms of reactions to different Plant growth regulators compounds.

Materials and methods

This study was conducted at a private tissue culture laboratory (Kurdistan Shinaran Company) located in Sanandaj, Kurdistan province from October 2014 to August 2015. First of all, 150 strawberry transplants of Kurdistan colony from 3 strawberry-prone areas (Sanandaj, Mariwan, and Kamyaran) with diverse weather conditions were collected (Table 1).

Table- 1- Climatic conditions 3 strawberry-prone area Kurdistan province

Area	Longitude	Latitude	Altitude	Mean annual rainfall	Climatic state and natural resources	An average annual maximum temperature	An average annual minimum temperature	Average annual heat
Kamyaran	46^0 and $54^{'}$	34^0 and $47^{'}$	1464 m	502 mm	Hot and moderate	25.9 ^{0}c	0.3 ^{0}c	12.9 ^{0}c
Sanandaj	46° and 59'	35° and 10'	1500 m	492 mm	Cold and moderate	44 ^{0}c	-13.5 ^{0}c	12.8 ^{0}c
Mariwan	46^0 and $45^{'}$	35^0 and $52^{'}$	1320 m	887 mm	Cold and moderate	36.7 ^{0}c	-8.4 ^{0}c	12.8 ^{0}c

Following that, the transplants were put in pots at lab's greenhouse. After putting them in pots and young Stolons production, and before making explants from leaf and meristem, the pots were treated through thermotherapy (38°C). Then, leaf and meristem explants were washed using dishwashing liquid and tap water and after three times of rinsing with distilled water, they were floated in 70% ethanol for 30 seconds in culture room. Subsequently, the explants were washed with distilled water for 3 times and were put in sodium hypochlorite 3% for 30 minutes and rewashed using distilled water for 3 more times [15] - [16].

One of the obstacles to explants growth is secretion of phenolic and tissue browning of tissues. Tissue browning starts some minutes after cutting explants and disinfection with sodium hypochlorite (generally known as bleach). To overcome this problem, 1% of Ascorbic acid, 0.2% of active coal or 2% of poly-vinyl-pyrrolidone (PVP) are used [17]. In this experiment, to solve the problem of browning tissues, 0.2% of active coal was used [18]. Finally, the explants were cultured on basic MS medium with different hormonal compounds and the samples were put in growth room (25±2°C with 16 hours of lightness and 8 hours of darkness).

Part 1: Examining the effects of growth regulators, explants, and the regions of samples collection on callus induction of Kurdistan colony strawberry. This part of experiment was done as a factorial in the form of Completely Randomized Designs (CRD) with 3 replications. The geographical regions of transplants collections including Mariwan, Sanandaj, and Kamyaran at three levels as factor A; cultured explants at two levels (meristem and leaf) as factor B and basic MS medium containing growth regulator compounds at 4 levels as factor C were considered. Factor C includes the following 4 levels: (a): a combination of 1 mg/l Thidiazuron (TDZ) and 0.2 mg/l 2, 4-D (b): a combination of 10 mg/l Benzyl amino purine (BAP) and 0.01 mg/l NAA (c): a combination of 2.65 mg/l Thidiazuron (TDZ) and 0.01 mg/l indole butyric acid (IBA) (d): MS medium without Plant growth regulators (control medium). Leaf and meristem explants

(extracted using Scalpel blades, forceps, and microscope) were cultured in vitro condition containing MS medium with four different Plant growth regulators compounds. After the culturing, the glasses were transferred to growth room (25±2°C with 16 hours of lightness and 8 hours of darkness). The used explants in this experiment were provided using two ways: 1) Young grown stolons in greenhouse. 2) Regenerated seedlings from meristem culture at in-vitro conditions. They had the same reactions in medium of experiment. In this part of the experiment, the following traits for callus were measured: percentage, wet weight, dry weight, growth rate, diameter in days 17, 23, 29, 35, transparency, color, stiffness and bump.

Part 2: This part of experiment carried out to investigation of callus organogenesis in the form of a Completely Randomized Designs (CRD) with 3 replications. Induced callus from the first part of the experiment were transferred to regeneration medium, which included the following three plant growth regulators compounds: 1) MS medium containing 2 mg/l of BAP in addition to 0.1 mg/l of NAA 2) MS medium containing 2 mg/l of IBA and 2 mg/l BAP 3) MS medium without Plant growth regulators (control medium). The following traits were measured at this part of the experiment: indirect regeneration, the number of regenerated cells, growth rate of regeneration, and the stem length in days 16, 22, 28, 34, and 40 and root length in day 40.

Part 3: examining the effect of 4 growth regulators on direct regeneration of Kurdistan colony meristem explants collected from 3 prone-strawberry regions including Kamyaran, Sanandaj and Mariwan, Kurdistan province, Iran. This part of experiment was conducted as a factorial in the form of Completely Randomized Designs (CRD) with 3 replications. Factor A included 3 main regions (Kamyaran, Sanandaj and Mariwan) that Kurdistan colony has extensively been cultivated for over half a century. Factor B included the following four hormonal compounds: (1): Basic MS medium containing a combination of 1 mg/l Thidiazuron (TDZ) and 0.2 mg/l 2.4.D (2): Basic MS medium containing a combination of 10 mg/l Benzyl amino purine (BAP) and 0.01 mg/l NAA (3): Basic MS medium containing a combination of 2.65 mg/l Thidiazuron (TDZ) and 0.01 mg /l indole butyric acid (IBA) (4): Basic MS medium without Plant growth regulators (control medium). Explants used in this part of the experiment included central bud meristem of young stolons in the greenhouse environment as well as regenerated plants meristem of in-vitro conditions. Before meristem separation, central buds disinfection was done based on the first section of experiment. Then, meristems were isolated under hood sterile and after that they were cultured at basic MS medium containing 4 plant growth regulators compounds. After explants are cultured, glasses were transferred to growth room (25±2°C with 16 hours of lightness and 8 hours of darkness). In this part, the following traits were measured: regeneration percentage, stem length in days 14, 21, 28, 35, root length in day 35, and regeneration growth rate.

Statistics analysis

The normality of the data in different parts was done using $SAS_{9.3}$ software. After we made sure that the assumptions of the variance analysis are true, analysis of variance and means comparison

was done using Duncan multiple range test at the level of 5%. Also, to draw the diagrams Excel software 2013 was used.

Results and Discussion

The first part of the experiment:
The leaf explants in all four growth regulators were completely destroyed ten days after the culture (Table 1). The driving force behind destroying leaf explants is the high rate of phenolic compounds of Kurdistan colony strawberry. In such conditions, leaf explants are not capable of dealing with high concentrations of phenolic compounds and are destroyed before callus induction, regeneration, and even modest growth (Figure 1). The results showed that there is a significant difference between two kind of explants (leaf and meristem) and the regions of collecting strawberry samples of Kurdistan colony in terms of callus induction. Mousavizadeh *et al* (2010), investigated callus induction and somatic embryogenesis of Camarosa cultivar in the combination of TDZ (12µM) + IBA (0.05µM) and BAP (44.4µM) + NAA (0.05µM) in leaf and petiole organs and observed that these two organs are capable of inducing callus [19]. Among meristem explants, only the meristem of Mariwan region in the combination of [BAP (10mg/l) + NAA (0.01mg/l)] had the capability of callus induction (Table 2). Meristem explants of Kamyaran and Sanandaj in all four tested plant growth regulators compounds were entered direct regeneration phase, i.e. they weren't capable of inducing callus (Figure 2) that indicated the role of selected explants for callus induction and genetic heterogeneity in valuable colony of Kurdistan strawberry.

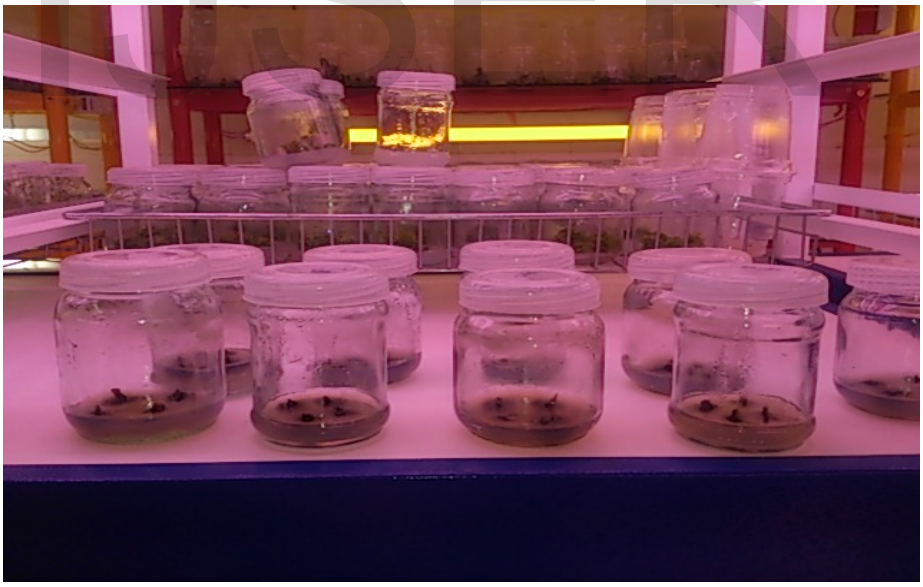

Figure-1: Leaf explants cultured in studied growth regulator compounds.

Figure-2: Induced callus from meristem explant of Mariwan region in growth regulator compounds: NAA (0.01mg/l) + BAP (10mg/l)

Alizadeh (2011) stated that the kind of explants and medium optimization are the main factors in successful callus induction [20]. The observed differences among studied regions (Kamyaran, Mariwan, and Sanandaj) in terms of callus induction can be an indicator of genetic heterogeneity in Kurdistan colony strawberry. Also, among employed plant growth regulators compounds the treatment of NAA + BAP was the best plant growth regulators treatment to induce callus in this colony, indicating the significant effect of plant growth regulators compounds on leading considered changes in tissue culture medium (Table 2).

The second part of the experiment (indirect regeneration):

Based on the analysis of variance, the following assessed traits were affected by plant growth regulators treatments ($P<0.01$): the percentage of indirect regeneration, the number of regenerated cells, growth regeneration rate, and stem length in days 16. 22, 28, 34, 40 and root length in day 40 (Table 3). The results indicated significant difference of administered plant growth regulators treatments at the probability level of $P<0.01$. Means comparison using Duncan multiple range test at the level of $P<0.05$ showed that out of two growth regulator treatments and a MS medium treatment (without growth regulator), studied in this experiment, two growth regulator treatments had the highest percentage of indirect regeneration and the highest number of regenerated cells. For other examined traits, however, treatment MS medium (without growth regulator) was higher than two growth regulator treatments (Table 4).

The third part of the experiment (direct regeneration):

Based on the results of variance analysis, the measured traits in this section of experiment were as follows: regeneration percentage, stem length in days 14, 21, 28, 35, root length in day 35 and

regeneration growth rate influenced by the growth regulators (hormones), regions ($P<0.05$) and the interaction effect of hormones × regions ($P<0.01$) (Table 5). This indicates the significant difference between different levels of growth regulators and the geographical regions which, in turn, shows different reaction of collected samples from various regions influenced by different types of plant growth regulators. The means comparison of assessed traits in this experiment was made using Duncan multiple range test ($P<0.05$). Based on the means comparison of hormones × regions interaction effect for germination percentage, we concluded that the best treatment for direct regeneration was related to growth regulator 2.4.D + TDZ in Mariwan region and the weakest one was in Kamyaran region (Tables 6, 7, and 8).

Table 2: The results of callus induction of meristem and leaf explants.

Area	Explant	Medium	Callus percent	Callus wet weigh (gr)	Callus dry weigh (gr)	Callus growth rate (cm/day)	Callus diameter (cm) day 17 (cm)	Callus diameter (cm) day 23	Callus diameter (cm) day 29	Callus diameter (cm) day 35	Transparency	Cohere	Callus color	Tension	Prominence
Kamyaran	Meristem	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0	0
	Leaf	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0	0
Sanandaj	Meristem	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0	0
	Leaf	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0	0
Mariwan	Meristem	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	77.77	0.451	0.131	0.039	0.327	0.549	0.794	0.966	transparent	coherent	amber	tense	prominence
		3	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0	0
	Leaf	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0	0

Medium 1: 2,4-D + TDZ

Medium 2: NAA + BAP

Medium 3: IBA + TDZ

Medium 4: Medium MS

Table 3: The Variance Analysis of plant growth regulators (Hormone) effects on indirect regeneration.

Sources of Variation	Df	Indirect regeneration	number of regenerated cells	Growth rate of regeneration	Stem length in day 16	Stem length in day 22	Stem length in day 28	Stem length in day 34	Stem length in day 40	Root length in day 40
Hormone	2	0.166**	877.777**	0.013**	0	0.731**	2.275*	4.680*	7.928**	10.346**
Error	6	0.0013	36.111	0.001	0	0.038	0.246	0.471	0.649	0.309
CV%	-	4.285	14.817	46.042	0	45.941	56.538	53.765	48.349	51.917

* and **: significant at the level of 5 and 1, respectively.

Table 4: Mean comparison of plant growth regulators tested on indirect regeneration using Duncan's test (P≤0.05)

Hormone	Indirect regeneration percent	number of regenerated cells	Growth rate of regeneration	Stem length in day 16	Stem length in day 22	Stem length in day 28	Stem length in day 34	Stem length in day 40	Root length in day 40
BAP(2mg/l)+NAA(0.1mg/l)	1[a]	55[a]	0.033[b]	0[a]	0.143[b]	0.386[b]	0.563[b]	0.69[b]	0[b]
BAP(2mg/l) + IBA(2mg/l)	1[a]	45[a]	0.031[b]	0[a]	0.14[b]	0.363[b]	0.55[b]	0.766[b]	0[b]
Medium MS	0.592[b]	21.667[b]	0.147[a]	0[a]	0.996[a]	1.883[a]	2. 72[a]	3.543[a]	3.216[a]

Means with the same letter are not significantly different.

Table 5: The variance Analysis of direct regeneration experiment.

Sources of Variation	df	Regeneration percentage	Stem length in day 14	Stem length in day 21	Stem length in day 28	Stem length in day 35	Root length in day 35	Growth rate of regeneration
Hormon	3	0.102**	0.092*	1.305**	3.50**	5.218**	6.204**	0.006**
Regions	2	0.034*	0.437**	1.831**	3.082**	6.764**	40.21**	0.008**
Hormon × Regions	6	0.261**	0.319**	2.229**	4.797**	8.397**	7.290**	0.010**
Error	24	0.008	0.026	0.087	0.162	0.228	0.171	0.0002
CV%	-	13.48	24.81	20.29	17.81	15.57	18.22	15.71

* and **: significant at the level of 5 and 1%, respectively.
Table numbers are mean squares.

Table 6: Means comparison of Kurdistan different regions in term of direct regeneration, using Duncan's multiple rang test (P≤0.05).

Regions	regeneration Percentage	Stem length in day 14	Stem length in day 21	Stem length in day 28	Stem length in day 35	Root length in day 35	Growth rate of regeneration
Kamyaran	0.703[a]	0.464[c]	1.030[b]	1.719[c]	2.224[b]	0.19[c]	0.079[b]
Sanandaj	0.703[a]	0.845[a]	1.56[a]	2.359[b]	3.334[a]	2.983[b]	0.118[a]
Mariwan	0.611[b]	0.664[b]	1.793[a]	2.72[a]	3.655[a]	3.636[a]	0.128[a]

Means with the same letter are not significantly different.

Table 7: Means comparison of plant growth regulators(Hormone) effects on direct regeneration using Duncan's multiple rang test (P≤0.05)

Hormone	Regeneration percentage	Stem length in day 14	Stem length in day 14	Stem length in day 14	length Stem in day 14	Root length in day 35	Growth rate of regeneration
TDZ (1mg/l) + 2.4,D (0.2mg/l)	0.740[a]	0.744[a]	1.694[a]	2.541[b]	3.32[b]	2.701[a]	0.118[b]
BAP(10mg/l)+NAA(0.01mg/l)	0.543[b]	0.515[b]	1.015[b]	1.516[d]	2.127[c]	1.163[c]	0.075[d]
TDZ(2.65mg/l)+IBA(0.01mg/l)	0.777[a]	0.713[a]	1.85[a]	2.96[a]	3.941[a]	3.071[a]	0.140[a]
Medium MS	0.658[a]	0.685[a]	1.285[b]	2.046[c]	2.895[b]	2.144[b]	0.101[c]

Means with the same letter are not significantly different.

The means comparison of geographical regions and growth regulator compounds interaction effects showed that the samples of Mariwan and Sanandaj regions in Plant growth regulators treatments [TDZ (1mg/l) + 2.4-D (0.2mg/l)] and [BAP(10mg/l) + NAA(0.01mg/l)] had the best regeneration percentage, respectively (Figure 3).

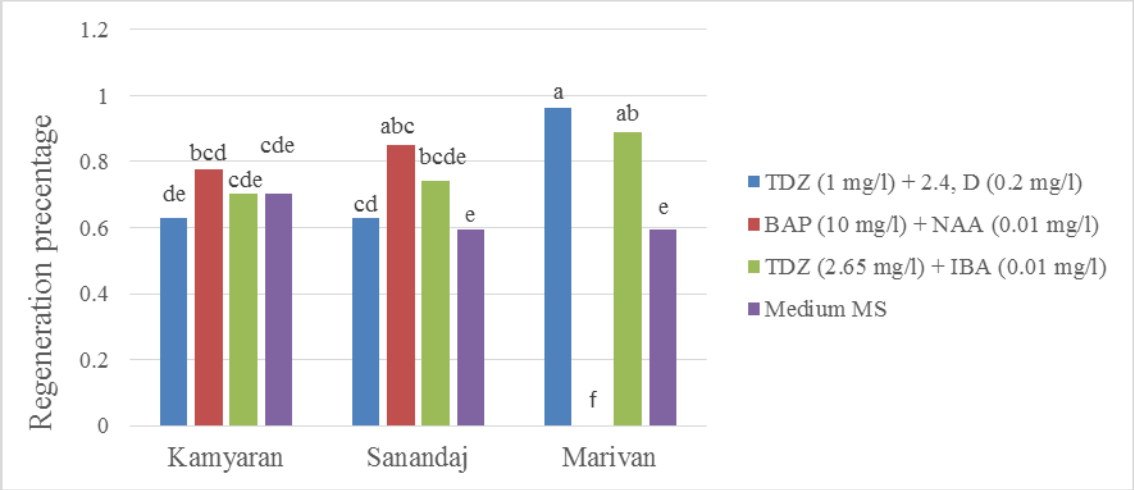

Figure 3: Means comparison of geographical regions and plant growth regulator interaction effects for Regeneration percentage in direct regeneration by Duncan's multiples test (P≤0.05).

We can also conclude that the best region for stem length in day 14 was related to collected samples from Sanandaj (Figure 4).

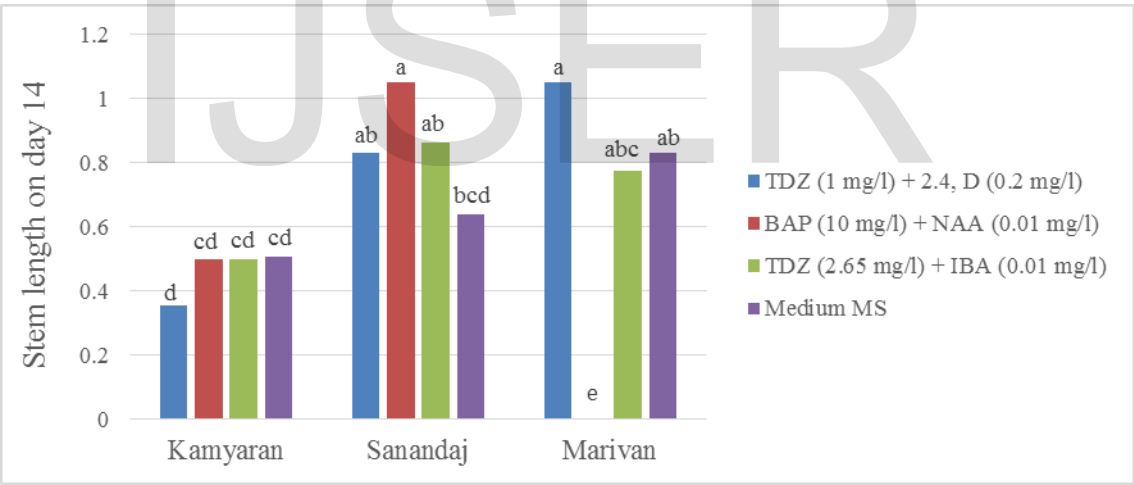

Figure 4: Means comparison of geographical regions and Plant growth regulators interaction effects for Stem length on day 14 in direct regeneration by Duncan's multiples test (P≤0.05).

Considering the mean comparison of interaction effects of growth regulator compounds with geographical regions, however, the weakest treatment compound in terms of stem length was belonged to Mariwan samples in Plant growth regulators [BAP(10mg/l) + NAA(0.01mg/l)]. This was because meristem explants of Mariwan samples in this Plant growth regulators treatment entered callus induction phase (Figures 4 & 5). The results, also, revealed that the weakest Plant growth regulators treatment was related to Plant growth regulators treatment [TDZ (1mg/l) + 2.4-D (0.2mg/l)] in Kamyaran (Figure 4).

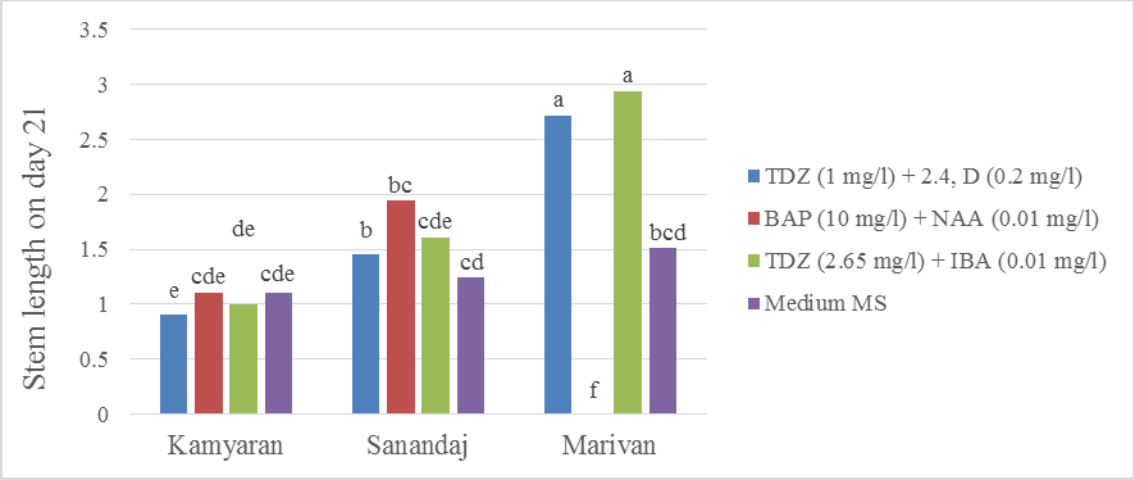

Figure 5: Means comparison of geographical regions and plant growth regulators interaction effects for Stem length on day 21 in direct regeneration by Duncan's multiples test (P≤0.05).

The diagram associated with the means comparison of geographical regions and plant growth regulator interaction effects depict that Mariwan sample in growth regulator IBA + TDZ showed the highest amounts of stem length in days 28 and 35 (Table 8; Figures 6 & 7). Too, the diagram associated with the means comparison of geographical regions and plant growth regulator interaction effects for root length in day 35 indicate that maximum and minimum root lengths in growth regulator IBA+TDZ were related to Mariwan and Kamyaran samples, respectively (Table 8; Figure 8).

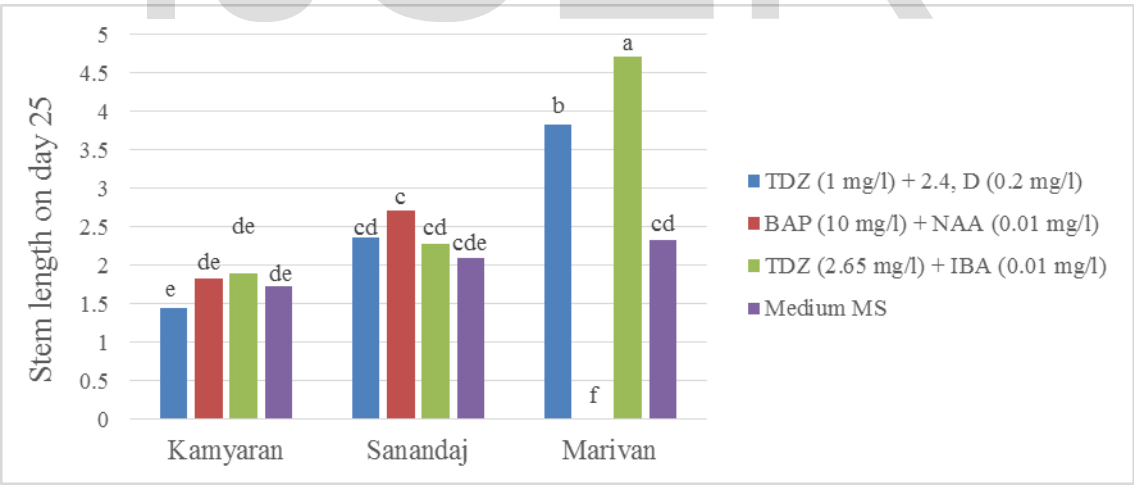

Figure 6: Means comparison of geographical regions and Plant growth regulators interaction effects for Stem length on day 28 in direct regeneration Duncan's multiples test (P≤0.05).

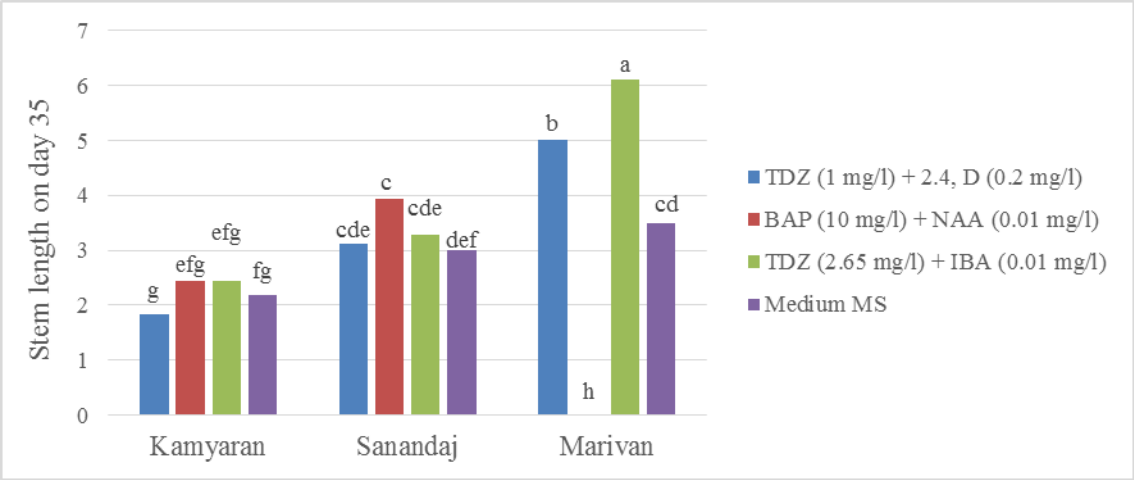

Figure7: Means comparison of geographical regions and Plant growth regulators interaction effects for Stem length on day 35 in direct regeneration by Duncan's multiples test (P≤0.05).

Based on means comparison of the hormones and regions interactions, it was found that the maximum and the minimum regeneration rate was related to Plant growth regulators compounds [IBA (0.01mg/l) + TDZ (2.65mg/l)] and [BAP (10mg/l) + NAA (0.01mg/l)] in meristem explants of Mariwan region, respectively. Also the minimum regeneration rate in Kamyaran belonged to [TDZ (1mg/l) + 2.4-D (0.2mg/l)] (Table 8). Meristem explants from Mariwan, in combination to [TDZ (2.65mg/l) + IBA (0.01mg/l)], was identified to be the best treatment compound for all investigated traits in direct regeneration experiment (Table 8). All in all, the results of the current study showed that direct and indirect regeneration rate of strawberry vary depending on genotype and Plant growth regulators treatments (Table 8).

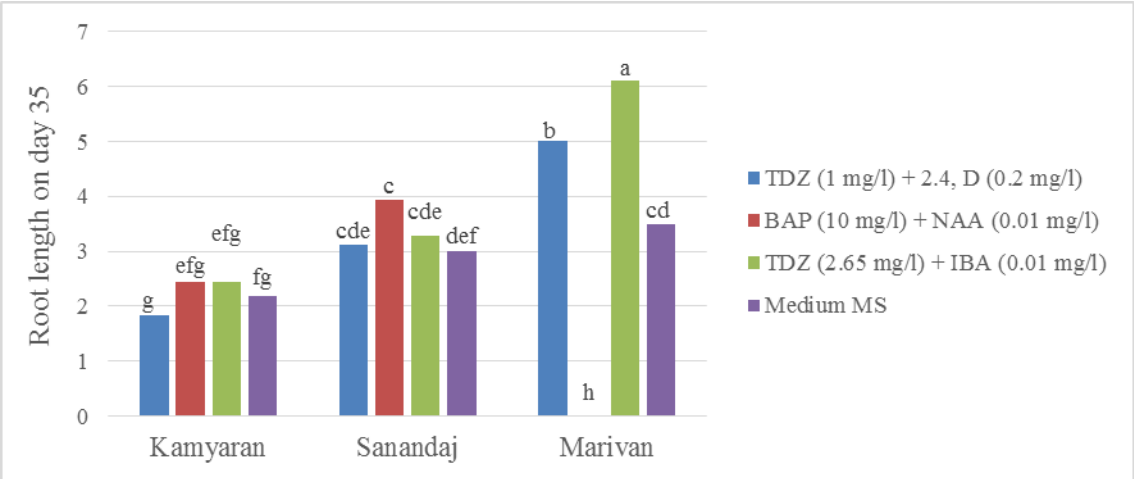

Figure 8: Means comparison of geographical regions and Plant growth regulators interaction effects for Root length on day 35 in direct regeneration by Duncan's multiples test (P≤0.05).

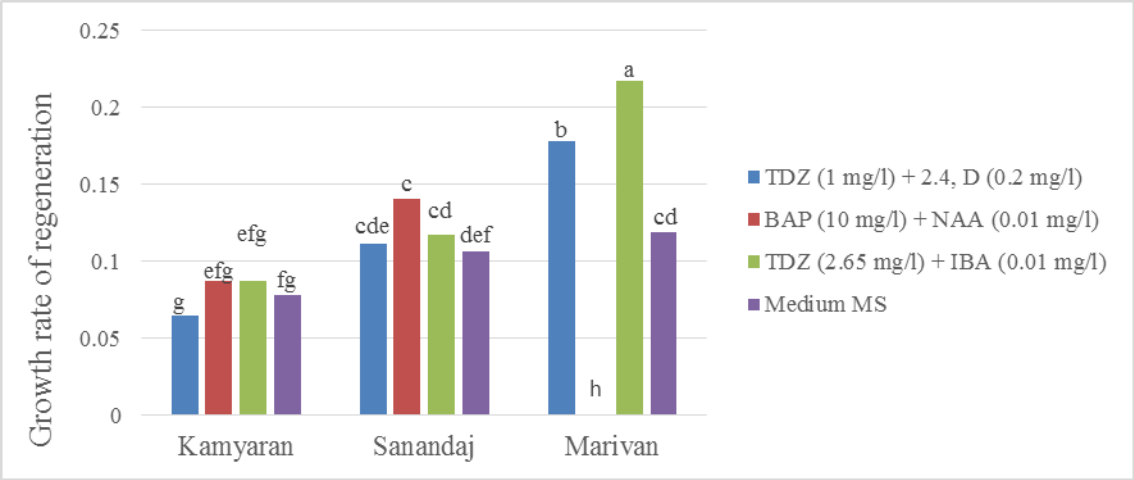

Figure 9: Means comparison of geographical regions and Plant growth regulators interaction effects for Growth rate of regeneration in direct regeneration by Duncan's multiples test (P≤0.05)

Figure10: Growth stages of strawberry (Meristem to Adult plant).

Table 8: Means comparison of geographical regions and plant growth regulators compounds interaction in direct regeneration experiment by Duncan's multiple range test (P≤0.05).

Regions	Hormone	Regeneration percentage	Stem length in day 14	Stem length in day 21	Stem length in day 28	Stem length in day35	Root length in day 35	Growth rate of regeneration
Kamyaran	**TDZ (1mg/l) + 2.4,D (0.2mg/l)**	0.692^{de}	0.353^{d}	0.91^{e}	1.44^{e}	1.83^{g}	0.163^{e}	0.065^{g}
	BAP(10mg/l)+NAA(0/01mg/l)	0.777^{bcd}	0.496^{cd}	1.106^{cde}	1.83^{de}	2.44^{efg}	0.16^{e}	0.077^{efg}
	TDZ(2.65mg/l)+IBA(0.01mg/l)	0.703^{cde}	0.5^{cd}	1^{de}	1^{de}	2.44^{efg}	0.22^{e}	0.087^{efg}
	Medium MS	0.703^{cde}	0.506^{cd}	1.106^{cde}	1.72^{de}	2.186^{fg}	0.216^{e}	0.077^{fg}
Sanandaj	**TDZ (1mg/l) + 2.4,D (0.2mg/l)**	0.692^{de}	0.83^{ab}	1.453^{b}	2.353^{cd}	3.12^{cde}	2.886^{cd}	0.111^{cde}
	BAP(10mg/l)+NAA(0.01mg/l)	0.851^{abc}	1.05^{a}	1.94^{bc}	2.72^{c}	3.943^{c}	3.33^{cd}	0.140^{c}
	TDZ(2.65mg/l)+IBA(0.01mg/l)	0.740^{bcde}	0.863^{ab}	1.606^{cde}	2.276^{cd}	3.276^{cde}	3.053^{cd}	0.117^{cd}
	Medium MS	0.592^{e}	0.64^{bcd}	1.24^{a}	2.086^{cde}	2.996^{def}	2.663^{d}	$0.106d^{ef}$
Mariwan	**TDZ (1mg/l) + 2.4,D (0.2mg/l)**	0.962^{a}	1.05^{a}	2.72^{a}	3.83^{b}	5.01^{b}	5.053^{b}	0.178^{b}
	BAP(10mg/l)+NAA(0.01mg/l)	0^{f}	0^{f}	0^{e}	0^{f}	0^{h}	0^{h}	0^{h}
	TDZ(2.65mg/l)+IBA(0.01mg/l)	0.888^{ab}	0.888^{ab}	2.943^{a}	4.716^{a}	6.106^{a}	5.94^{a}	0.217^{a}
	Medium MS	0.592^{e}	0.83^{ab}	1.51^{bcd}	2.333^{cd}	3.503^{cd}	3.553^{d}	0.118^{cd}

Means with the same letter are not significantly different.

Conclusion

- Leaf explants of the three studied regions were not able to grow in 4 hormonal compounds and were completely destroyed. The highest percentage of callus induction in meristem explants of Kurdistan colony strawberry belonged to collected ones in Mariwan, obtained from [BAP(10mg/l) + NAA(0.01mg/l)].
- Means comparison showed that in terms of regeneration percentage and the number of regenerated cells, MS medium containing [NAA (0.1 mg/l) + BAP (2 mg/l)] as well as plant growth regulators compound of [IBA (2 mg/l) +BAP (2mg/l)] was higher than medium (without hormone). However, regarding regeneration growth rate, stem length in days 22, 28, 34, 40, and root length indirect regeneration experiment, MS medium (without hormone) was higher than the other two compounds.
- The highest percentage of direct regeneration among 4 studied plant growth regulators compounds belonged to plant growth regulators compound [2.4.D (0.2mg/l) + TDZ (1mg/l)]. In terms of other plant growth regulators compounds, however, the highest one was related to [IBA (0.01mg/l) + TDZ (2.65mg/l)]. Among the three studied geographical regions, the highest amount of measured traits belonged to collected samples of Mariwan.
- The results showed that the most appropriate region for callus induction and direct regeneration was Mariwan, in which the best plant growth regulators compound for callus induction was [NAA (0.01mg/l) + BAP (10mg/l)] and the best hormonal compound for direct regeneration was IBA (0.01mg/l) + TDZ (2.65mg/l)].
- Strawberry colony, in this study known as Kurdistan colony in prone-strawberry regions including Sanandaj, Mariwan, and Kamyaran, is heterogeneous and is genetically diverse. This claim could be proved through highly significant differences among explants from collected samples in terms of callus induction, organogenesis of induced callus, and direct regeneration. Therefore we hope that through a comprehensive morphological, biochemical, and molecular study of this colony in different regions of Kurdistan province, better and more favorable regions, out of this genetically heterogeneous colony, are identified and as a result a new colony with unique characteristics (such as excellent taste and aroma) is introduced.

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